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**A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF ANATOMY

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**By
HAROLD B. KENTON**

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SPECIES SPECIFICITY OF FIBRINOGENS

HAROLD B. KENTON

From the Laboratory of Preventive Medicine, The University of Chicago

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INTRODUCTION

The selectivity with which antibodies react with the particular proteins used in their production affords a biological method of differentiating proteins which is far more delicate than that of direct chemical analysis.

Applications of this method, in one form or another, have been made by various workers in an attempt to determine whether the blood protein, fibrinogen, is an identical protein in all animal species or is in each particular instance a protein peculiar to that species.

The conclusions reached by the several investigators are contradictory and the purpose of this paper is to present experiments which offer more extensive proof that fibrinogens are species specific, as are other blood proteins.

In 1912 Bauer and Engel (1) reported that beef and swine fibrinogens exhibited individual and species specificity when tested by the method of complement fixation. They also used the precipitin reaction but found that specificity was not so definitely demonstrable by this method. Kato (2) in 1922, on the other hand, found that immune serum prepared against mammalian fibrinogen acted not only on the homologous fibrinogen but also on the fibrinogen of other mammalian species. He stated that an immune serum gained by injecting rabbits with a foreign mammalian fibrinogen did not react with rabbit fibrinogen but that rabbits injected with rabbit fibrinogen produced an immune serum that reacted with other mammalian fibrinogens although not with rabbit fibrinogen.

Hektoen and Welker (3), working with a large number of different mammalian fibrinogens, also reported, on the basis of the precipitin reaction, a decided lack of species specificity. These authors stated that not only are the mammalian fibrinogens not strictly species specific but that their tests indicated some relationship between mammalian and domestic fowl fibrinogen.

Kyes and Porter (4) prepared an immune serum against fowl fibrinogen. This serum caused precipitation of fowl fibrinogen but did not precipitate either sheep or horse fibrinogen. In 1932 Demanez (5) tested anti-swine fibrinogen against horse, beef and sheep fibrinogens by the precipitin method. This author reported that "the quantitative differences are sufficient to totally exclude the hypothesis of one and the same fibrinogen in the case of different mammals."

METHODS

In all the investigations cited above, the crucial antibody experiments were performed *in vitro*, either as precipitin or as complement fixation tests. In my own investigations, on the other hand, I have preferred as a basal criterion the *in vivo* antibody-antigen reaction which induces anaphylactic shock. The preference is based upon the belief that this reaction, under the conditions imposed, is more constant, more delicate and more reliable than are the *in vitro* reactions.

The general program has been the sensitization of series of animals with a given fibrinogen and the determination of whether or not such animals were sensitive to the fibrinogens of other species. To this has been added observations as to the specificity displayed in desensitization.

As experimental animals I have employed pigeons, which animals as pointed out by Gahringer (6), are especially favorable for observations of anaphylaxis. The relatively large leg veins afford a favorable avenue both for bleeding and for quantitative intravascular injection and the ability of these animals almost invariably to survive shock favors systematic study over a long period. Also the constancy with which pigeons may be sensitized and the uniformity of their symptoms of shock favors their use for this type of experimentation.

In duly sensitized pigeons, the shock reaction is, as described by Kyes and Strauser (7), an acute reaction of relatively short duration but having distinctive symptoms. Within from one to four minutes fol-

lowing injection of the "critical" dose, the average reacting animals show ptosis, lachrymation, mucous discharge from the nose and mouth, sneezing and dyspnea: A little later respiration becomes extremely rapid and the animals become "wobbly" and sink to the floor if the reaction is severe. The symptoms quickly abate until, at the end of half an hour, the animals usually appear normal or at most but slightly drowsy. Death may occur but is infrequent.

The various fibrinogens used were prepared from fresh oxalate plasma. To each 100 cc. of plasma, 25 grams of sodium chloride (c.p.) were added. The mixture was stirred for ten minutes and the resulting fibrinogen precipitate was removed by centrifugalization. The precipitate was redissolved in a 3 per cent solution of sodium chloride containing 0.1 per cent potassium oxalate. Three to four reprecipitations were made and the final fibrinogen precipitate was dissolved in 3 per cent sodium chloride containing 0.1 per cent potassium oxalate. The volume of the final solution of fibrinogen was one-half the volume of the original plasma and will be referred to as the "standard" solution. The fibrinogens of five different species were used in the experiments.

EXPERIMENTS

Experiment I. A group of thirteen normal pigeons was injected intravenously, each with 1 cc. of the "standard" solution of sheep fibrinogen diluted 1:10. Fourteen days after such sensitizing injection, seven of these birds were injected intravenously with 1 cc. of "standard" sheep fibrinogen (homologous) and the remaining six with 1 cc. of "standard" horse fibrinogen (heterologous). The degree of anaphylactic shock resulting from these "critical" injections is given in table 1.

The results given in table 1 show that of the thirteen pigeons sensitized with sheep fibrinogen, all seven which received a critical injection of sheep fibrinogen displayed anaphylactic shock, whereas, all six receiving horse fibrinogen as a critical injection failed to show shock.

The same results were obtained in the following experiment in which a larger number of animals was used.

Experiment II. Eighteen normal pigeons were sensitized by an intravenous injection of 1 cc. of "standard" sheep fibrinogen diluted 1:10. After twelve days, eight of these animals received

intravenously a critical dose of "standard" sheep fibrinogen (homologous) while the remaining ten received a similar dose of horse fibrinogen (heterologous). The degree of shock induced is given in table 2.

As recorded in table 2 the results of experiment II show that of eighteen pigeons sensitized with sheep fibrinogen, those eight which received a critical dose of sheep fibrinogen all showed anaphylactic shock, whereas, the ten receiving horse fibrinogen showed no shock.

TABLE 1

Pigeons sensitized with sheep fibrinogen; reaction to sheep and horse fibrinogens

PIGEON NUMBER	CRITICAL INJECTION OF FIBRINOGEN FROM	DEGREE OF REACTION*
408	Sheep	Moderate
401	Sheep	Moderate
405	Sheep	Moderate
407	Sheep	Moderate
423	Sheep	Moderate
410	Sheep	Slight
406	Sheep	Moderate
412	Horse	0
424	Horse	0
413	Horse	0
416	Horse	0
418	Horse	0
420	Horse	0

* *Marked* signifies severe dyspnea plus the inability of the animal to stand; *moderate*, a marked dyspnea; *slight*, ptosis usually accompanied by slight respiratory disturbance; and *0*, no reaction.

From these experiments it may be concluded that antibodies produced to sheep fibrinogen do not react with horse fibrinogen and hence that the fibrinogens of the two species are not identical but dissimilar in structure.

The following experiment shows that pigeons sensitized to sheep fibrinogen not only fail to react to horse fibrinogen but also to domestic fowl fibrinogen.

Experiment III. Eighteen normal pigeons were injected intravenously, each with a sensitizing dose of 1 cc. of "standard"

sheep fibrinogen diluted 1:10. Fifteen days later these birds were divided into three groups and were given critical intravenous injections as follows: Six animals received 1 cc. of "standard" sheep fibrinogen (homologous); six others received a similar dose of horse fibrinogen (heterologous) and the remaining six received fowl fibrinogen (heterologous). The degree of reaction resulting in the three groups is given in table 3.

TABLE 2

Pigeons sensitized with sheep fibrinogen; reaction to sheep and horse fibrinogens

PIGEON NUMBER	CRITICAL INJECTION OF FIBRINOGEN FROM	DEGREE OF REACTION
66	Sheep	Slight
59	Sheep	Slight
69	Sheep	Moderate
68	Sheep	Moderate
64	Sheep	Moderate
58	Sheep	Moderate
52	Sheep	Slight
55	Sheep	Marked
60	Horse	0
65	Horse	0
57	Horse	0
53	Horse	0
63	Horse	0
61	Horse	0
56	Horse	0
62	Horse	0
67	Horse	0
54	Horse	0

In table 3 it is seen that pigeons sensitized to sheep fibrinogen not only fail to react to a critical dose of horse fibrinogen, as in the two previous experiments, but fail equally to react with domestic fowl fibrinogen, allowing the conclusion that the fibrinogens of sheep and fowl are dissimilar.

The following experiment is parallel to those given above except that the heterologous fibrinogen employed was that of the rabbit.

Experiment IV. Sixteen normal pigeons were given a sensitizing dose of 1 cc. of "standard" sheep fibrinogen diluted 1:10.

After twelve days eight of the animals received a critical dose of 1 cc. of "standard" sheep fibrinogen (homologous) and the remaining eight the same dose of rabbit fibrinogen (heterologous). The reaction of these animals is given in table 4.

The above experiment shows that pigeons sensitized with sheep fibrinogen fail to react with rabbit fibrinogen.

The combined experiments thus far presented show that pigeons when sensitized to sheep fibrinogen do display constant anaphy-

TABLE 3

Pigeons sensitized with sheep fibrinogen; reaction to sheep, horse and chicken fibrinogens

PIGEON NUMBER	CRITICAL INJECTION OF FIBRINOGEN FROM	DEGREE OF REACTION
958	Sheep	Moderate
976	Sheep	Moderate
974	Sheep	Moderate
967	Sheep	Marked
973	Sheep	Moderate
952	Sheep	Moderate
961	Horse	0
970	Horse	0
964	Horse	0
969	Horse	0
957	Horse	0
963	Horse	0
977	Chicken	0
953	Chicken	0
978	Chicken	0
954	Chicken	0
966	Chicken	0
974	Chicken	0

lactic shock when injected with a critical dose of sheep fibrinogen but do not display shock when injected with a critical dose of the fibrinogens derived from horse, domestic fowl or rabbit. Upon this basis the conclusion is made that antibodies produced in pigeons sensitized with sheep fibrinogen, fail to react with the fibrinogens of horse, fowl and rabbit because sheep fibrinogen is not identical with that of any of the other three species named.

The results thus far considered leave unanswered the question

as to whether the dissimilarity established for sheep fibrinogen, may by the same method be established for a fibrinogen other than sheep. The following experiment (V) shows that this may be done.

Experiment V. Eighteen normal pigeons were sensitized by an intravenous injection of 1 cc. of "standard" beef fibrinogen diluted 1:10. Fifteen days after the sensitizing injection, eight of the animals received intravenously a critical injection of 1 cc. of the "standard" beef fibrinogen (homologous) while the re-

TABLE 4

Pigeons sensitized with sheep fibrinogen; reaction to sheep and rabbit fibrinogens

PIGEON NUMBER	CRITICAL INJECTION OF FIBRINOGEN FROM	DEGREE OF REACTION
40	Sheep	Slight
43	Sheep	Slight
31	Sheep	Moderate
30	Sheep	Moderate
45	Sheep	Marked
29	Sheep	Moderate
49	Sheep	Moderate
27	Sheep	Marked
34	Rabbit	0
39	Rabbit	0
35	Rabbit	0
38	Rabbit	0
33	Rabbit	0
41	Rabbit	0
36	Rabbit	0
46	Rabbit	0

maining ten animals were given a similar injection of rabbit fibrinogen (heterologous). The results of this experiment are presented in table 5.

From table 5 it may be observed that when pigeons are sensitized with beef fibrinogen they display a shock reaction upon the critical injection of beef fibrinogen but display no reaction upon injection of rabbit fibrinogen. A dissimilarity in structure is thus shown to exist also between beef and rabbit fibrinogens.

A further demonstration of the dissimilarity of fibrinogens was

made by employing the phenomena of anaphylaxis in a modified form.

It is well known that an animal, sensitized to a given protein, may be desensitized to that protein by a parenteral injection of the same. For desensitization, the dose of the homologous protein need not be sufficient to produce a noticeable shock reaction in the animal. Desensitization is not produced by a corresponding dose of a protein other than that used in the sen-

TABLE 5

Pigeons sensitized with beef fibrinogen; reaction to beef and rabbit fibrinogens

PIGEON NUMBER	CRITICAL INJECTION OF FIBRINOGEN FROM	DEGREE OF REACTION
24	Beef	Moderate
22	Beef	Moderate
25	Beef	Slight
9	Beef	Moderate
20	Beef	Slight
18	Beef	Moderate
16	Beef	Moderate
7	Beef	Slight
1	Rabbit	0
11	Rabbit	0
6	Rabbit	0
2	Rabbit	0
21	Rabbit	0
3	Rabbit	0
5	Rabbit	0
8	Rabbit	0
17	Rabbit	0
12	Rabbit	0

sitization and the selectiveness of this action is the basis of the following experiment.

Experiment VI. Eighteen pigeons were sensitized to beef fibrinogen by the intravenous injection of 1 cc. of the "standard" solution diluted 1:10. After a period of twelve days, eight of these birds received an estimated desensitizing dose of beef fibrinogen and the remaining ten a corresponding dose of rabbit fibrinogen. The desensitizing doses were given intravenously. Sixteen hours after these injections, all the pigeons were given a

critical injection of 1 cc. of "standard" beef fibrinogen intravenously. The results are shown in table 6.

The above experiment which shows a contrast in the desensitizing value of beef and rabbit fibrinogens gives further proof that these fibrinogens are not identical.

In the general line of experimentation which I have pursued to demonstrate that the fibrinogens of all species are not identical,

TABLE 6

Pigeons sensitized with beef fibrinogen; reaction to beef fibrinogen after desensitization

PIGEON NUMBER	DESENSITIZED WITH FIBRINOGEN FROM	REACTION TO CRITICAL DOSE OF BEEF FIBRINOGEN
18	Beef	0
22	Beef	0
7	Beef	0
25	Beef	0
16	Beef	0
9	Beef	0
24	Beef	0
20	Beef	0
12	Rabbit	Moderate
1	Rabbit	Marked
3	Rabbit	Moderate
8	Rabbit	Slight
6	Rabbit	Slight
5	Rabbit	Moderate
17	Rabbit	Marked
2	Rabbit	Moderate
11	Rabbit	Moderate
21	Rabbit	Moderate

I have purposely employed comparisons of fibrinogens from species only remotely related zoologically. Beginning with the classical work of Nuttall (8), repeated demonstration has been made that the species specificity of the antibody-protein reactions although of a very high order is not absolute, namely that antibodies produced to the proteins of a given species may to a certain degree react with the proteins of a closely related species. Since such "group" antibody-protein reactions are observable in the case of the domestic ruminants I have for completeness made

comparisons of the fibrinogens of closely related species such as beef and sheep. The results of such experiments show that pigeons sensitized with sheep fibrinogen react not only to a critical dose of sheep fibrinogen but, also, of beef fibrinogen. In the fibrinogens of closely related species, therefore, there exists sufficient similarity of structure to afford the "group" antibody reactions such as occur with the other proteins of such species.

CONCLUSION

On the basis of the preceding experiments which employ as a differential method the specificity of the sensitization of animals to a foreign protein, I conclude that fibrinogens are not of identical protein structure in all species, but are dissimilar to the extent that their differences in structure are demonstrable except as they may occur between closely related species.

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